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Methods for Conducting Snail (Aplexa hypnorum) Embryo through Adult Exposures Effects of Cadmium and Reduced pH Levels

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Methods for Conducting Snail (*Aplexa hypnorum*) Embryo Through Adult Exposures: Effects of Cadmium and Reduced pH Levels

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Abstract. Two separate embryo through adult exposures were conducted with cadmium and with reduced pH levels to validate various test methodologies and to determine the feasibility of testing and ease of handling the freshwater snail (*Aplexa hypnorum*) in a test system designed for fish bioassays. Exposure of snails from embryos through adult reproductive maturity to cadmium chloride produced delayed hatch, reductions in percentage hatch and survival, and reduced growth when compared to a control. Based on these effects, the maximum acceptable toxicant concentration in Lake Superior water was between 4.41 and 7.63 μg cadmium/L in one test and between 2.50 and 4.79 μg cadmium/L in another test. Adult snails were exposed to determine a 96-hr LC50 of 93 μg cadmium/L. Exposure of embryonic, larval and adult snails to a pH range of 5.00–5.75 caused delayed hatching, reduced combined hatchability and survival, reduced 26-day growth, and a reduction in the number of egg masses per adult snail. No adverse effects were observed in snails exposed for 26 days to pH values ranging from 6.25 to 6.65. During a short-term static exposure, a pH of 4.3 caused a cloudy appearance in eggs within the egg mass and killed the embryos within two hr of the start of the exposure. A static pH exposure of 4.6 also turned embryos slightly cloudy, although some development did occur in these embryos by the sixth day.

Snails used in all tests were *Aplexa hypnorum* which are members of the family, *Physidae*. This family of snails is found world-wide and *Aplexa* is circumboreal. Aplexa have been collected from swamps or muskeg, roadside ditches, vernal ponds and intermittent streams, temporarily flooded marginal areas near permanent streams, large to medium sized rivers, large lakes, and occasionally in small slow-flowing streams (Clarke 1973). *Aplexa* are monoecious with facultative self- and cross-fertilization. The egg mass contains a gelatinous substance of a viscous jellied nature which holds the eggs in place, and as hatch commences, turns to a more fluid consistency. The egg and egg mass serve a dual purpose of providing a source of nutrition and protection for the embryos. *Aplexa* was chosen as the test species, because populations of snails were readily available from tanks of a fathead minnow (*Pimephales promelas*) holding system at the Environmental Research Laboratory-Duluth (ERL-D) and because these snails have a rapid life-cycle (~30 days) at 25°C. Several methods of handling egg masses were used to determine which method was most advantageous.

Cadmium chloride and various levels of reduced pH were tested using snail embryos, larvae and adults through reproductive maturity to allow the determination of an estimated maximum acceptable toxicant concentration (MATC). These MATC values were based on observed effects on percentage hatch, survival, growth and/or reproduction in exposed snails. In addition to life-cycle tests, a cadmium acute test using adult snails and a short-term (6-day) reduced pH test with snail embryos were conducted to determine sensitivity differences between short- and long-term exposures.

Cadmium was tested because it is a cumulative poison that is a common water contaminant, and there is no chronic cadmium toxicity information available for snails. Acute information is available for cadmium and various species of snails (Ravera 1977; Rehwoldt *et al.* 1973; Gupta *et al.* 1981; Wier

and Walter 1976; Spehar *et al.* 1978). Spehar *et al.* (1978) also determined a 28-day cadmium LC50 for *Physa integra*.

Effects of reduced pH were determined, since the lowering of pH levels due to acid rain and acid mine drainage are an increasing concern for the aquatic environment. The European Inland Fisheries Advisory Commission (EIFAC 1969) summarized the literature regarding effects of lowered pH on aquatic organisms and suggested the need for information on long-term effects of reduced pH levels. Garve (1980) previously described the effects of depressed pH on survival, growth, reproduction and egg development of the pond snail, *Helisoma trivolis*. Several investigators (Walton and Wright 1926; Okland and Okland 1980) have also described the presence and distribution of other freshwater snails in relation to hydrogen ion concentration.

Results of embryo, larval and adult tests with *Aplexa* were compared to chronic data in the literature for fish species to determine any large or consistent sensitivity differences. This was done to determine whether snails are generally more or less sensitive to cadmium and reduced pH than fish species.

Materials and Methods

Exposure System

Exposure systems for these tests consisted of modified 2 L proportional diluters (Mount and Brungs 1967). Dilution factors were 0.6 for cadmium and varied in the pH test to achieve the desired pH levels. Diluter modifications included addition of an electronic timer, microswitch and water solenoids to regulate cycle time and use of simplified siphons and flow-splitting chambers (Benoit and Puglisi 1973). During each diluter cycle, these flow-splitting chambers divided each of the six exposure levels to quadruplicate exposure tanks. The letters A, B, C and D were used to designate each of the four individual sets of six tanks in the quadruplicate series. The 24 glass exposure tanks measured 50.0 × 14.5 × 15.0 cm deep with a 10.0 cm standpipe, which yielded a tank volume of 7.3 L. Standpipes had 40 mesh nylon screen sleeves attached to prevent newly hatched snails from escaping from the tanks. Flow rates and 90% replacement time of the test water, determined by the method of Sprague (1969), were 56 ml/min and 4.6 hr and 100 ml/min and 2.6 hr for the cadmium and pH tests, respectively. Tanks were not cleaned or siphoned after the snails hatched to preclude loss or injury of the snails. A 16-hr photoperiod was used. Fluorescent lamps provided a light intensity of 28 lumens at the water surface.

Water Characteristics

Test water was obtained from Lake Superior and heated to a mean test temperature of 23.9 ± 0.6°C. Dissolved oxygen (D.O.) concentrations were measured in one series of quadruplicate tanks once each week using a dissolved oxygen meter (calibrated by the azide modification of the Winkler method). Means and ranges of D.O. measurements were 8.5 (7.3–9.3) and 7.9 (7.5–8.2) mg/L in the cadmium and pH tests, respectively. All other water characteristics were measured by methods described by the American Public Health Association (1980). Total hardness, alkalinity, and pH determinations in the cadmium tests and total hardness determinations in the pH test were made once each week on three tanks of one quadruplicate series (always including a control and high concentration tank). The mean and range of all total hardness and alkalinity measurements were 45.3 (43.2–48.0) and 40.3 (39.0–41.8) mg/L as $CaCO_3$, respectively. The pH in the cadmium tests ranged from 7.3 to 7.6. Alkalinity and acidity were altered in the pH test by additions of hydrochloric acid, and these parameters were measured in one series of the quadruplicate tanks (6 tanks) once each week. Means and ranges for alkalinity measurements were 44.4 (42.5–45.8), 32.0 (31.0–33.5), 21.3 (19.7–22.7), and 5.0 (3.9–5.9) mg/L as $CaCO_3$ in tanks with mean pH readings of 7.74 (control), 6.98, 6.48 and 5.45, respectively. Alkalinity was not measurable in tanks with mean pH levels of 5.00 and 3.07. Means and ranges for acidity were 1.3 (0.5–2.1), 5.9 (2.3–8.3), 12.9 (10.7–15.9), 22.5 (20.0–24.7), 23.4 (18.7–28.1) and 72.3 (65.5–83.7) mg/L as $CaCO_3$ in tanks that had mean pH values of 7.74, 6.98, 6.48, 5.45, 5.00 and 3.07, respectively.

Toxicant Solutions

Anhydrous cadmium chloride ($CdCl_2$) was obtained from Matheson, Coleman and Bell[1]. The cadmium salt was dissolved in Lake Superior water and metered into the diluter during each diluter cycle. Water samples were taken from the quadruplicate tanks so that cadmium concentrations were measured in all tanks once each week. Cadmium was analyzed with a Perkin-Elmer 5000 atomic absorption spectrophotometer equipped with an HGA 500 graphite furnace and an AS-40 auto-sampler. Instrument settings were those recommended by the manufacturer. Method 213.2 of EPA Methods for Chemical Analysis of Water and Wastes (U.S. Environmental Protection Agency 1979) was used. Spike recoveries in control water averaged 99.1 ± 3.6% (N = 28). A quality assurance sample (true value = 1.50 µg/L) from the EPA Environmental Monitoring and Support Laboratory in Cincinnati was analyzed to be 1.47 ± 0.06 (N = 26). The detection limit was approximately 0.20 µg/L. Means and standard deviations of cadmium measurements are given in Table 1.

Reagent grade hydrochloric acid (38% HCl) used during the pH exposure was obtained from Baker Chemical Company[2]. The desired pH range in the test tanks was achieved by slowly dripping 3N HCl from a Marriotte bottle into the diluter mix cell at a constant rate. The pH of all test tanks (Table 2) was monitored daily, Monday through Friday, using a calibrated pH meter.

Biological Procedures

Embryo, Larval, and Adult Exposures: Egg masses used to start the 26-day exposures were obtained from an Environmental Re-

[1] Matheson, Coleman and Bell, Norwood, OH 45212

[2] J. T. Baker Chemical Co., Phillipsburg, NJ 08865

Table 1. Survival, growth, and egg mass production in snails exposed to various concentrations of cadmium in A, B and C, D duplicate tanks

Measured concentrations (μg/L)	Combined percentage[a] hatchability and survival	Total number of snails in the two duplicates surviving until termination	Average shell length of all snails exposed at each concentration (mm)	Total number of egg masses in tanks at termination	Number of egg masses per snail (#egg masses)/(# snails)
			A and B Tanks		
Control (<0.1)	86.9 ± 3.2[b]	74	5.5 ± 0.6[b]	97	1.3
1.52 ± 0.13[b]	62.6 ± 35.5	62	5.5 ± 1.1	75	1.2
2.41 ± 0.28	83.8 ± 13.2	65	5.6 ± 1.2	81	1.2
4.41 ± 0.76	62.5 ± 2.5	68	5.2 ± 1.6	56	0.8
7.63 ± 0.60	11.9 ± 8.7*	11	3.5 ± 0.9*	0	—
13.2 ± 0.70	1.1 ± 1.5*	1	3.6*	0	—
			C and D Tanks		
Control (<0.1)	92.7 ± 3.7[b]	95	4.9 ± 1.0[b]	106	1.1
1.51 ± 0.27[b]	85.0 ± 8.1	58	5.8 ± 1.4	106	1.8
2.50 ± 0.30	41.7 ± 16.6	39	4.9 ± 2.0	31	0.8
4.79 ± 0.74	35.0 ± 33.3*	29	2.6 ± 0.8*	0	—
7.17 ± 0.72	3.4 ± 4.7*	3	2.6 ± 0.6*	0	—
12.9 ± 0.56	0 ± 0	0	—	0	—

[a] This was derived by dividing the number of snails surviving until termination by the number of embryos that showed apparent normal development four days after starting the tests

[b] Mean ± standard deviation of the combined duplicate tanks

Asterisk (*) denotes values significantly less than controls (analysis of variance and Dunnett's procedure, P = 0.05)

search Laboratory-Duluth (ERL-D) holding system used to provide fathead minnows and adult snails for acute testing. Adult snails lay numerous egg masses on the sides and ends of these 30 × 30 × 60 cm glass holding tanks. The sides and ends of these tanks were thoroughly cleaned 24 hr before starting all tests to ensure that all egg masses were between 0 and 24 hr old. Egg masses were gently dislodged and suctioned into a 100-ml volumetric pipet which had the tip removed. Egg masses were randomly mixed before they were selected for testing. In the cadmium test, each egg mass was placed in the top of a 100 × 15 mm disposable polystyrene petri dish together with 10 ml of water and then the bottom of the petri dish was inverted over the egg mass. This procedure compressed the egg mass into one plane so the embryos could be easily counted with a dissecting microscope. Each bright reflection under the egg mass on the bottom of the petri dish represented one egg. Two egg masses containing 20 or more embryos were placed on the clean tank bottoms of the twelve A and B duplicate exposure tanks and in oscillating cups made from 0.12 L glass jars with 40 mesh nylon screen bottoms in the twelve C and D duplicate tanks. The number of embryos in each egg mass used in the pH test was carefully counted using a dissecting microscope, but without compressing the egg mass. Egg masses containing between 20 and 30 embryos were selected and placed in the large half of a polystyrene petri dish. A petri dish containing one egg mass was gently filled with tank water and allowed to sink to the bottom of all exposure tanks. This procedure made it easier to find, remove and return the egg mass to the tank during observations on development. Embryo development was checked beginning on the fourth day of the cadmium test, fifth day of the pH test, and daily thereafter until the egg mass liquefied and hatch had begun. At the start of hatch, egg cups were submerged on their sides on the bottom of their respective cadmium C and D exposure tanks to allow escape of snails consistent with other methods of handling egg masses. Hatch was not quantified, since hatch occurred over a period of several days and since egg masses were not handled during hatch to prevent injury or loss of young snails. Egg masses in exposure tanks where delayed embryo development was observed were monitored daily until hatch began or the embryos died. Beginning at first observed hatch, snails in each tank were fed four drops of concentrated live brine shrimp twice a day on weekdays and once a day on weekends. After one week, feeding was increased to 1 ml of concentrated brine shrimp per tank twice a day on weekdays and 2 ml once per day on weekends. Both tests were terminated after 26 days and within 4 days after the first egg masses were observed in any exposure tank to prevent any newly spawned embryos from hatching. At 26 days, all surviving snails in each tank were measured for total shell length and the number of egg masses present in each tank was recorded. In several instances in the pH tests where all the embryos were killed, newly hatched snails (from extra egg masses placed in an egg cup in a control tank at the start of the test) were introduced into the tanks to observe if they would survive and grow at pH levels that killed embryos. Fifty-two, 47, and 18 newly hatched snails were added to exposure tanks with pH ranges of 3.00–3.25, 4.40–5.65, and 4.60–5.65, respectively.

Acute Testing—Cadmium: A flow-through 96-hr acute cadmium test was conducted with adult snails. This test was conducted with the same diluter system (five concentrations plus a control) and flow rates as described earlier for the cadmium embryo through adult exposure. Ten snails were counted into 10 cm × 3 cm wide stainless steel screen (20 mesh) cylinders which were closed on one end by stainless steel screen and on the other end by a #7 neoprene stopper. Cylinders containing the snails were

Table 2. Survival, growth and egg mass production in snails exposed to various pH levels

Quadruplicate tank	pH range	Total number of snails surviving until termination	Combined percentage[a] hatchability and survival of snails in each exposure tank	Combined percentage hatchability and survival of snails in the combined quadruplicate tanks	Total number of snails surviving until termination in the quadruplicate tanks	Average shell length of all snails surviving until termination in the quadruplicate tanks	Total number of egg masses in the quadruplicate tanks at termination	Number of egg masses per snail (#egg masses)/(# snails)	Mean and range of pH measurements of combined quadruplicate tanks
1A	7.50–7.90	7	100.0	88.9 ± 10.0	62	7.1 ± 1.2	200	3.2	7.74 (7.50–8.00)
1B	7.50–7.90	19	79.2						
1C	7.50–8.00	18	81.8						
1D	7.50–7.90	18	94.7						
2A	6.80–7.25	11	52.4	74.0 ± 15.6	55	7.2 ± 0.8	164	3.0	6.98 (6.75–7.25)
2B	6.80–7.20	12	75.0						
2C	6.80–7.10	17	89.5						
2D	6.75–7.20	15	78.9						
3A	6.35–6.65	9	75.0	90.5 ± 10.3	76	7.0 ± 0.8	192	2.5	6.48 (6.25–6.65)
3B	6.25–6.60	22	95.7						
3C	6.30–6.60	19	95.0						
3D	6.30–6.60	26	96.3						
4A	5.10–5.65	11	52.3	68.5 ± 14.6[c]*	45	6.6 ± 1.3*	96	2.1*	5.45 (5.00–5.75)[c]
4B	4.60–5.65	—[b]	0						
4C	5.05–5.75	21	80.8						
4D	5.00–5.75	13	72.2						
5A	4.50–5.60	—[b]	0	0 ± 0*	0	—	0	—	5.00 (3.90–5.65)
5B	4.40–5.65	—[b]	0						
5C	4.00–5.35	—[b]	0						
5D	3.90–5.35	—[b]	0						
6A	3.00–3.20	—[b]	0	0 ± 0*	0	—	0	—	3.07 (3.00–3.25)
6B	3.00–3.25	—[b]	0						
6C	3.00–3.25	—[b]	0						
6D	3.00–3.25	—[b]	0						

[a] Derived by dividing the number of snails surviving until termination by the number of embryos that showed apparent normal development five days after starting the test

[b] All embryos died before hatching

[c] Tank 4B was not included in this mean since the pH range in this tank was similar to those in tanks 5A through 5D and all of the snails died

Asterisk (*) denotes values significantly less than controls (analysis of variance and Dunnett's procedure, $P = 0.05$)

randomly distributed to exposure tanks that contained mean cadmium concentrations of 0.0, 72.6, 119, 174, 332, and 593 μg/L. Snail mortality was recorded at 96 hr. Means and ranges for total hardness and alkalinity were 44.8 (43.6–46.1) and 40.7 (39.9–41.3) mg/L as $CaCO_3$, respectively. The mean and range for dissolved oxygen measurements was 7.5 (7.3–7.9) mg/L while the pH ranged from 7.4 to 7.5. Snails were not fed during acute tests.

Short-Term pH Test: During the embryo through adult pH test, rapid coagulation of egg protein and a cloudy appearance of some embryos was observed. To more accurately determine what pH will affect embryogenesis, a series of ten beakers containing 500 ml of Lake Superior water were titrated with 3N HCl to pH values of 3.1, 3.6, 4.0, 4.3, 4.6, 4.9, 5.1, 5.2, 5.5 plus a control. One egg mass (0–24 hr old) was placed into each beaker and maintained at 25°C for 6 days. Observations were made at 1, 2, 19, 24 hr and again at 6 days when embryos began to hatch.

Statistical Analysis

Since exact hatchability values were not quantified, a combined percentage hatchability and survival was derived by dividing the number of snails that survived to termination by the number of embryos that showed apparent normal development at 4 or 5 days after the embryo through adult exposures were started. Non-viable eggs or embryos with little or no shell development at 4 or 5 days were considered abnormal or dead and were not included in combined hatchability and survival calculations. These combined percentage hatchability and survival data were normalized by using an arcsin square root transformation procedure described by Bartlett (1947). Normalized hatchability and survival data and snail growth data were subjected to one-way analysis of variance with an F-test of significance ($P = 0.05$) and to subsequent analysis with Dunnett's one-sided comparison of treatment means to control means ($P = 0.05$) (Steel and Torrie 1960). The A, B duplicates and C, D duplicates in the cadmium test were analyzed separately, since they were conducted by slightly different methods (A and B = egg masses exposed on tank bottoms, and C and D tanks = egg masses exposed in egg cups). Growth data from A, B duplicates and C, D duplicates in the cadmium test and from all quadruplicate tanks in the pH tests were combined before conducting the analysis of variance. Determination of the acute 96-hr LC50 for cadmium was made by the Trimmed Spearman Karber method (Hamilton *et al.* 1977).

Results

Cadmium

Eighty-four per cent of the snail eggs added initially to all cadmium concentrations and controls showed apparent normal development after 4 days. Hatch of snail embryos occurred from 6 to 9 days after initiation of the test in controls and cadmium concentrations up to and including 4.79 μg/L. Egg masses in higher cadmium concentrations turned to a fluid consistency and broke up during the same period of time as those in the lower concentrations and controls; however, hatch was delayed by cadmium concentrations of 7.17 μg/L and above. No hatching was observed in embryos exposed to 7.17 μg cadmium/L and above at 10 days, and many embryos remained unhatched 12 days after initiation of the test. No hatch occurred in cadmium concentrations of 12.9 μg/L, although one embryo did hatch and survive until termination at 13.2 μg/L (Table 1).

In the A, B duplicate tanks, the percentage hatch and survival and the average shell length of snails at termination were significantly reduced by cadmium concentrations of 7.63 and 13.2 μg/L (Table 1). Percentage hatch and survival and growth based on shell lengths were significantly reduced at cadmium levels of 4.79 μg/L and above in the C, D duplicates. No egg mass production was observed in any exposure tank which had significant effects on snail survival and growth. The number of egg masses per snail in the exposure tanks at termination was not significantly ($P = 0.05$) decreased by 4.41 μg cadmium/L and below (Table 1). The MATC for snails exposed in the A and B tanks, based on delayed hatch, reduced hatch and survival, and on reduced growth, lies between 4.41 and 7.63 μg cadmium/L. The MATC in the C and D tanks, based on a reduction in the percentage hatch and survival and on reduced growth, lies between 2.50 and 4.79 μg cadmium/L.

Cadmium Acute

The 96-hr LC50 value and 95% confidence interval for cadmium and adult snails was 93 (54–160) μg/L.

pH Test—Embryo through Adult

Disregarding tanks where all added embryos were killed, 81% of the total number of snail eggs added initially to all pH levels showed apparent normal development after 5 days. No snails hatched in the two lowest pH levels tested (Table 2). A pH of 3.00–3.25 (range of quadruplicate tanks) caused egg protein precipitation within 1.5 hr and the center portion of these eggs was completely white. The pH range of the next set of quadruplicate tanks (3.90–5.65) also caused a cloudy appearance of the eggs at 5 days after the start of the test. This cloudy appearance made it impossible to observe any embryo development and none of these cloudy eggs hatched. All eggs were also cloudy in appearance and died before hatching in tank 4B which had a pH range of 4.60–5.65 (Table 2). The remaining three tanks of this quadruplicate series had a pH

range of 5.00–5.75. Embryos exposed to this pH range turned slightly cloudy, but development did occur, although hatching was delayed and occurred between 9 and 12 days. Snail hatch at all higher tested pH levels and in controls occurred between 6 and 9 days after starting the test.

The combined percentage hatchability and survival and growth, based on shell lengths, were significantly reduced in snails exposed to pH levels ranging from 5.00–5.75 (Table 2). Exposure of snails to a pH range of 5.00–5.75 also significantly reduced the number of egg masses per snail observed in the tanks at termination (Table 2). Based on these effects, pH values within a range of 5.00–5.75 in Lake Superior water have definite adverse effects on populations of *Aplexa hypnorum*. No apparent adverse effects were observed at pH ranges of 6.25–6.65 and above (Table 2).

Added Snails

Survival of snails, added as newly hatched snails until termination, was 0, 31.9, and 72.2% at pH exposures of 3.00–3.25, 4.40–5.65 and 4.60–5.65, respectively. The mean and standard deviation of shell length measurements made on added snails that survived until termination at pH ranges of 4.40–5.65 and 4.60–5.65 were 5.9 ± 0.6 mm and 6.3 ± 0.8 mm, respectively. These growth values are both significantly less ($P = 0.05$) than the growth values for snails in unaltered Lake Superior water (pH = 7.50–8.00).

pH Static

There was an immediate white coloration in eggs exposed to pH levels of 3.1 and 3.6 and these eggs were dead within one hr. Eggs exposed to pH levels of 4.0 and 4.3 were beginning to have a cloudy appearance after one hr and were dead within two hr. Eggs turned slightly cloudy after two hr at pH levels of 4.6 and 4.9, and after 19 hours at pH 5.1. Exposure to pH levels of 5.2 and above did not affect the appearance of snail eggs. Some development was observed in snail embryos at a pH of 4.6 and hatch commenced at pH levels of 4.9 and above after 6 days of exposure.

Discussion

There are no cadmium toxicity data available in the literature for *Aplexa hypnorum*, although several acute studies have been conducted with cadmium and other species of freshwater snails. Ravera (1977) tested both adults and embryos of the pulmonate snail, *Biomphalaria glabrata*, and found embryos to be more resistant than adults to low cadmium concentrations. He determined that 50% survival times (ST-50) were 15, 31 and 63 hr for adult snails and 0.5, 2.5 and 8.0 days for embryos at cadmium concentrations of 1,000, 500 and 100 μg/L, respectively. Apparently, adult *Biomphalaria glabrata*, with a ST-50 of 63 hr at 100 μg cadmium/L (Ravera 1977), are equivalent or slightly more sensitive than *Aplexa* which had a 96-hr LC50 of 93 μg/L during the present tests. All other tested species are more resistant to cadmium than *Aplexa*. Rehwoldt *et al.* (1973) exposed eggs and adult snails, *Amnicola* sp., to cadmium and found the eggs (96-hr LC50 of 3,800 μg/L) to be more sensitive than adults (96-hr LC50 of 8,400 μg/L). A similar cadmium 96-hr LC50 value of 8,400 μg/L was determined for the Indian freshwater pond snail, *Viviparus bengalensis* (Gupta *et al.* 1981). Cadmium 96- and 228-hr LC50 values for mature snails, *Physa gyrina*, and the 96-hr LC50 for immature snails were determined (Wier and Walter 1976) to be 1,370, 830 and 410 μg/L, respectively. Spehar *et al.* (1978) calculated a 7-day LC50 of 114 μg/L for cadmium-exposed snails, *Physa integra*.

No life-cycle toxicity tests were found in the literature for cadmium and freshwater snails, although Spehar *et al.* (1978) determined a 28-day LC50 for *Physa integra*. These investigators found the 28-day LC50 of 10.4 μg/L was approximately 11 times lower than the 7-day cadmium LC50 of 114 μg/L. During the present test, adverse effects were observed in *Aplexa* embryos, larvae and adults at cadmium levels of 7.63 μg/L in the A, B duplicate tanks and at 4.79 μg/L in the C, D tanks. These effect levels are 12.2 and 19.4 times lower than the 96-hr LC50 of 93 μg/L for this species. Apparently, long-term snail tests and especially life-cycle exposures show adverse effects of cadmium at much lower levels than do acute tests.

Aplexa were much less sensitive in chronic tests than the other invertebrate species (*Daphnia magna*) tested with cadmium. Biesinger and Christensen (1972) determined chronic limits for cadmium exposed *Daphnia* of 0.17–0.7 μg/L.

The chronic MATC limits derived for cadmium and 11 species of freshwater fish (U.S. Environmental Protection Agency 1980) ranged from 0.9–1.34 μg/L for chinook salmon, *Oncorhynchus tshawytscha* (Chapman 1975) to 31.0–80.0 μg/L for bluegills (Eaton 1974). Three fish species were slightly more sensitive to cadmium and eight species were less sensitive than *Aplexa*.

Results of the snail embryo, larval, and adult pH exposures are presented and discussed as a range of pH values instead of mean values since there was some variability of pH readings in the test tanks, and it may have been the lower pH values that produced the observed effects. A pH range of 5.00–5.75 caused significant adverse effects on *Aplexa*, while a pH range of 6.25–6.65 produced no observable adverse effects during present testing. In another test, Garve (1980) studied the effects of reduced pH on survival, growth, reproduction, and egg hatching success in snails, *Helisoma trivolvis* and found similar effect levels. He found the growth rates of adults were inhibited at pH 5.65, egg production and egg hatching success were reduced at pH levels below 5.65 and concluded the safe pH for populations of these snails was above 6.0. Other investigators have looked at the distribution and presence of other species of snails with regard to hydrogen ion concentration. Walton and Wright (1926) studied the distribution of *Limnaea* common in North Wales and observed *L. truncatula* occurred over a pH range of 6.0 to 8.6 and *L. peregra* over a pH range of 5.8 to 8.8. In a study of 1,000 Norwegian lakes, Okland and Okland (1980) observed that freshwater snails (Gastropoda, as a group) are generally not found below pH 6.0, although a few species occurred at low density levels down to pH 5.2 if the calcium concentration was not low. Results of the present pH static test with snail egg masses also indicate that a short-term exposure (<2 hr) to a pH of 4.3 or less, during a possible acid spill, could seriously impact a population of *Aplexa*.

The EIFAC (1969) made a critical review of published and unpublished data on both direct and indirect effects of extreme pH values on freshwater fish. They concluded that there was no definite pH range within which a fishery is unharmed and outside which it was damaged, but rather there is a gradual deterioration as the pH values are further removed from the normal range. The pH range which is not directly lethal to fish is 5–9, although an acid discharge may liberate sufficient carbon dioxide from bicarbonate in the water, either to be directly toxic, or to cause the pH range of 5–6 to become lethal (EIFAC 1969). During chronic fish exposures to reduced pH, Mount (1973) found fathead minnow egg production and egg hatchability were reduced at pH 5.9, and Menendez (1976) noted that growth and survival of brook trout (*Salvelinus fontinalis*) alevins were reduced at pH 6.5. Generally, *Aplexa* are similar to freshwater fish in sensitivity to lowered pH, although brook trout appear slightly more sensitive.

The hatching process of snail eggs was delayed and growth was significantly reduced by cadmium concentrations of 7.17 μg/L and above, and by pH levels of 5.00–5.75. Since all tanks were terminated on the 26th day during both tests, the delayed hatching process may have accounted for some of the growth effects that occurred in these tanks.

Several new methods were used during the snail embryo, larval, and adult exposures. Live brine shrimp were used as a food source since *Aplexa* thrive in holding tanks for fish that are fed only brine shrimp. The snails apparently feed on the carcasses of the dead shrimp, although snails may have been feeding to some extent on slight algae growths that occurred in the unscrubbed tanks. Snails were fed the same amount of brine shrimp during both the cadmium and pH exposures even though there were more snails present in the cadmium exposure tanks. This probably accounts for the fact that snails were smaller at termination in the cadmium test than in the pH test. Snails in the cadmium exposure reached sexual maturity and began producing egg masses at approximately the same time as snails in the pH exposure, although the number of egg masses produced per snail was less in the smaller cadmium-exposed snails. Egg masses used to start the cadmium exposure were compressed into one plane to allow easy and accurate counting of the embryos. Although this procedure would be recommended for larger egg masses it is not necessary for egg masses containing 30 or less embryos and was not used during the pH test. One egg mass per quadruplicate tank was exposed in the pH test, while two egg masses were used in each cadmium tank since different procedures of exposing egg masses were used in the A, B and C, D duplicate tanks. During all tests, four egg masses were tested at each exposure level with a given method. Based on the number of embryos showing normal development after 4 or 5 days, this number of egg masses appears adequate for testing. Exposing egg masses in a submerged petri dish (pH test) was the best of the three exposure methods used. This method allowed easy location of the egg mass, easy removal for observations of development, and allowed hatched snails an easy, immediate escape into the exposure tanks. Exposing egg masses on the tank bottom (cadmium A and B tanks) often made locating the egg masses difficult. Use of oscillating egg cups (cadmium C and D tanks) facilitated finding and removing egg masses but could possibly hinder the escape of hatched snails into the tanks. However, the differences in MATC values observed between the A, B and C, D duplicate tanks were probably influenced more by slight differences in cadmium concentrations (Table 1) than by differences in exposure methods.

There is a general shortage of long-term toxicity data available for aquatic invertebrate species which are often important parts of the food web. *Aplexa hypnorum* is a good invertebrate species for embryo, larval and adult testing, because this snail is relatively simple to work with (even in a test system designed for use with fish), has a rapid life-cycle, and appears similar in sensitivity to fish in exposures to cadmium and reduced pH.

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References

American Public Health Association, American Water Works Association, Water Pollution Control Federation (1980) Standard methods for the examination of water and wastewater. 15th ed. Washington, DC

Bartlett MS (1947) The use of transformation. Biometrics 3:39

Benoit DA, Puglisi FA (1973) A simplified flow-splitting chamber and siphon for proportional diluters. Water Res 7:1915

Biesinger KE, Christensen GM (1972) Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. J Fish Res Board Can 29:1691

Chapman GA (1975) Toxicity of copper, cadmium, and zinc to Pacific Northwest salmonids. U.S. Environmental Protection Agency, Corvallis, OR

Clarke AH (1973) The freshwater molluscs of the Canadian Interior Basin. Malacologia 13

Eaton JG (1974) Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). Trans Amer Fish Soc 4:729

European Inland Fisheries Advisory Commission (1969) Water quality criteria for European freshwater fish—extreme pH values and inland fisheries. Water Res 3:593

Garve JC (1980) The effects of depressed pH on the survival, growth, reproduction, and egg development of the pond snail *Helisoma trivolvis*. Masters Thesis, University of Minnesota, Duluth, MN

Gupta PK, Khangarot BS, Durve VS (1981) Studies on the acute toxicity of some heavy metals to an Indian freshwater pond snail *Viviparus bengalensis* L. Arch Hydrobiol 91:259

Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman-Karber method for estimated median lethal concentrations in toxicity bioassays. Environ Sci Technol 7:714. Correction, 12:417 (1978)

Menendez R (1976) Chronic effects of reduced pH on brook trout (*Salvelinus fontinalis*). J Fish Res Board Can 33:118

Mount DI (1973) Chronic effect of low pH on fathead minnow survival, growth, and reproduction. Water Res 7:987

Mount DI, Brungs WA (1967) A simplified dosing apparatus for fish toxicology studies. Water Res 1:21

Okland J, Okland KA (1980) Acidification threatens trout diet-studies of 1000 lakes in Norway. University of Oslo, P.O. Box 1027, Blindern, N-Oslo 3, Norway

Ravera O (1977) Effects of heavy metals (cadmium, copper, chromium, and lead) on a freshwater snail: *Biomphalaria glabrata* Say (Gastropoda, Prosobranchia). Proc Fifth Europ Malac Congr Malacologia 16:231

Rehwoldt R, Lasko L, Shaw C, Wirhowski E (1973) The acute toxicity of some heavy metal ions toward benthic organisms. Bull Environ Contam Toxicol 10:291

Spehar RL, Anderson RL, Fiandt JT (1978) Toxicity and bioaccumulation of cadmium and lead in aquatic invertebrates. Environ Pollut 15:195

Sprague JG (1969) Measurement of pollutant toxicity of fish. 1. Bioassay methods for acute toxicity. Water Res 3:793

Steel RGD, Torrie JH (1960) Principles and procedures of statistics with special reference to the biological sciences. New York: McGraw-Hill

U.S. Environmental Protection Agency (1979) Method for chemical analysis of water and wastes. EPA-600/4-79-020, Cincinnati, OH

——— (1980) Ambient water quaiity criteria for cadmium. EPA-440/5-80-025. Office of Water Regulation and Standards, Criteria Standards Division, Washington, DC

Walton CL, Wright WR (1926) Hydrogen-ion concentration and the distribution of *Limnaea truncatula* and *L. peregra*, with a note bearing on mosquitoes. Parasitology 18:24

Wier CF, Walter WM (1976) Toxicity of cadmium in the freshwater snail, *Physa gyrina* Say. J Environ Qual 5:359

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